Functional Organization of Crayfish Abdominal Ganglia: I. The Flexor Systems

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Abstract:

For insect ganglia, Altman *(Advances in Physiological Science, Vol. 23. Neurobiology of Invertebrates.* New York: Pergamon Press, pp. 537-555, '81) proposed that individual neuropils control different motor activities. A corollary of this hypothesis is that motor neurons involved in many behavioral functions should branch in more neuropils than those active in fewer behaviors. In crayfish, the abdominal fast-flexor muscles are active only during the generation of the powerstroke for tailflips, whereas the slow-flexor muscles are involved in the maintenance of body posture. The slow flexors are thus active in many of the crayfish's behavioral activities. To test the generality of Altman's idea, we filled groups of crayfish fast-flexor and slow-flexor motor neurons with cobalt chloride and described their shapes with respect to the ganglionic structures through which they pass. Individual fast flexors were also filled intracellularly with HRP. Ganglia containing well-filled neurons were osmicated, embedded in plastic, and sectioned. Unstained sections were examined by light microscopy and pertinent sections were photographed. We found that the paths of the larger neurites were invariant, that the dendritic domains of fast and slow motor neurons occupied distinctive sets of neuropils, and that dendrites of slow motor neurons branched in more ganglionic structures than did those of fast motor neurons. These results are consistent with Altman's hypothesis.

Key words: axon tract, backfill, commissure, neuropil, motor neurons

Abbreviations

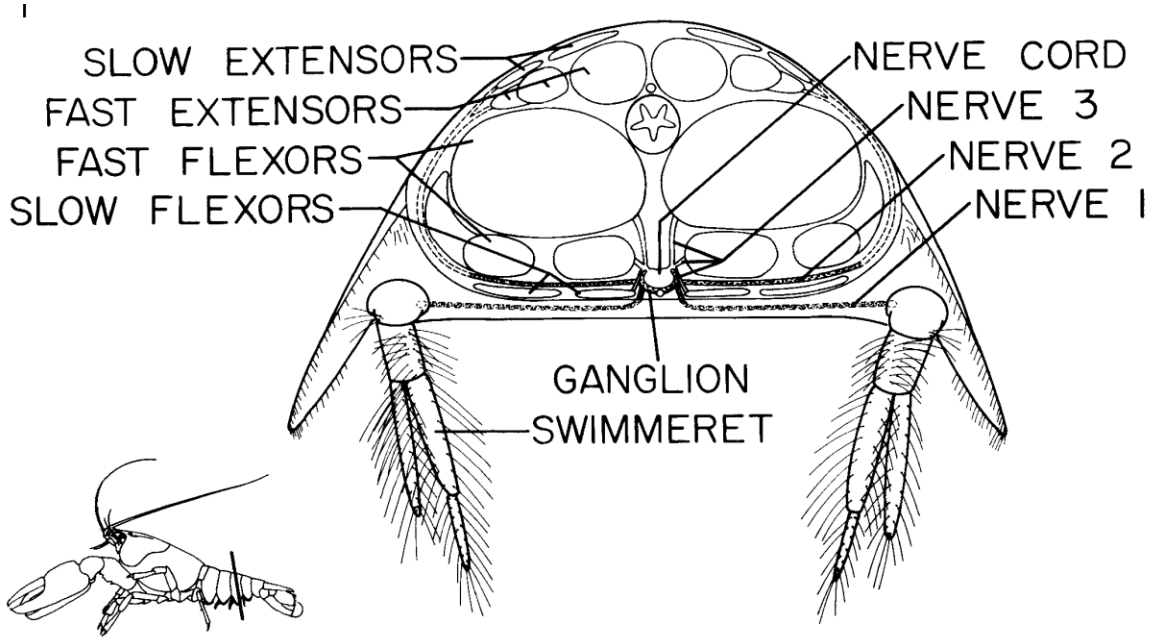
AMN	anterior midline neuropil
AVC	anterior ventral commissure
DC#	dorsal commissures (numbered 1–7)
DIT	dorsal intermediate tract
DLT	dorsal lateral tract
DMT	dorsal median tract
HN	horseshoe neuropil
LDT	lateral dorsal tract
LG	lateral giant axon
LN	lateral neuropil
LVT	lateral ventral tract
MDT	median dorsal tract
MG	medial giant axon
MoG	motor giant neuron
MVT	median ventral tract
N#	nerve 1, 2, or 3
PMN	posterior midline neuropil
PVC	posterior ventral commissure
RN2	root of nerve 2
RT	ring tract
TN	tract neuropil
VAC	ventral association center
VIT	ventral intermediate tract
VLT	ventral lateral tract
VLT_i	inner ventral lateral tract
VLT_o	outer ventral lateral tract
VMT	ventral median tract

Article:

The concept that vertebrate brains are constructs of coordinated but functionally unique regions was established in the last century (reviewed in Changeux, '83; Shepherd, '83) and has been applied more recently to invertebrates (Bullock and Horridge, '65). Arthropod brains have distinct lobes or neuropils subserving different sensory functions (Williams, '75; Strausfeld and Nassel, '80; Ernst and Boeckh, '83). Evidence is now

accumulating that the thoracic and abdominal ganglia also contain neuropils with unique sensory and motor roles (Tyrer and Altman, '74; Romer, '83; Murphey, '85; Johnson and Murphey, '85; Murphey et al., '85; Paul and Mulloney, '85; Skinner, '85a,b; Wohlers and Huber, '85).

Altman (Altman and Kien, '79; Altman, '81) stressed the importance of ganglionic integrative centers and proposed that in insect ganglia, different sensory and motor neurons project to different areas of neuropil depending upon the function of these neurons (i.e., flight or walking). She concluded that different motor activities would be controlled by different neuropils. Coordination of the assorted motor activities into complex behaviors would occur by means of neurons that integrate information from several regions. We suggest that such ganglionic organization is not limited to insects, but among the larger invertebrates, is a cornerstone of nervous system function.



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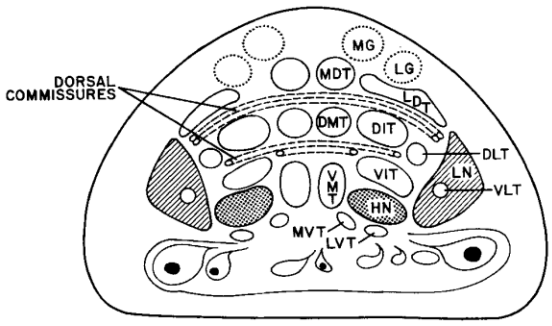

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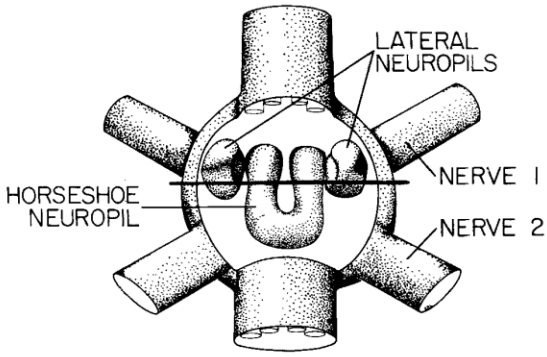


Fig. 1. Diagram of the nerves and muscles within an abdominal segment of *Pacifastacus leniusculus*. The view is toward the animal's rostrum. The nerves and nerve cord are enlarged for clarity. Nerve 2 passes caudad and out of the plane of this section. However, its general direction toward the target muscles has been indicated as dashed lines. Nerves 1 and 2 also carry sensory axons, but the starting points of these sensory axons have been omitted. Inset: Drawing of a crayfish. The line through the abdominal segment 4 indicates the plane of section used to draw the diagram (modified from Bishop, et al., '84).

Fig. 2. Diagram of a midganglionic section, illustrating the positions of major longitudinal axonal tracts (solid ovals), commissures, neuropils, and giant fibers (dotted circles). Somata are ventral. Approximate plane of section through the ganglion is represented by the line in Figure 3. Anterior and posterior structures (e.g., anterior ventral commissure, anterior midline neuropil, and the curve of the horseshoe neuropil) are absent from this plane of section.

Fig. 3. Diagram of spatial relationship between three largest discrete neuropils within abdominal ganglia 2–5, drawn with a dorsal exposure and as if all other internal ganglion structures had been removed. The arms of the HN extend more anteriorly and ventrally than do the LNs. The unlabeled line indicates the approximate plane of section drawn in Figure 2.

To test the generality of Altman's ('81) hypothesis, we marked two functionally distinct populations of motor neurons in crayfish by backfilling their axons with cobalt chloride and by filling individual motor neurons with horseradish peroxidase. We then traced the paths of these neurons through the identified tracts, commissures, and neuropils (Skinner, '85a) in each abdominal ganglion.

In our study, we exploited the functional segregation of the three pairs of nerves that exit from each abdominal ganglion in the crayfish. The abdominal ganglia control three types of motor activities: rhythmic beating of the abdominal appendages (the swimmerets), rapid tailflips during backward swimming, and maintenance of abdominal posture. Axons in each nerve, or in each major branch of a nerve, are associated with only one of these motor activities. The anterior pair of nerves (Nerves 1) innervates the swimmerets and contains motor and sensory axons (Fig. 1). The motor functions of these nerves are concerned only with swimmeret movement. Nerves 2 innervate the slow-and fast-extensor muscles, stretch receptors, and sensory hairs of the body wall (Fig. 1). The motor functions of these nerves are twofold: the slow extensors are active in postural control while the fast extensors generate the returnstroke of tailflips. The superficial and main branches of Nerves 3 innervate the slow- and fast-flexor muscles, respectively (Fig. 1) (Kennedy and Takeda, '65a,b). The slow flexors are active in the control of posture and the fast flexors in the generation of the tailflip powerstroke. The six types of abdominal muscles (fast and slow extensors and flexors, and power- and return-stroke swimmeret muscles) perform motor activities that are variously combined into complex behaviors. For example, the swimmerets beat during ventilation, forward swimming (mostly in juveniles), and righting behavior. In all of these behaviors, the slow extensors and flexors keep adjacent segments in their appropriate relative positions. The fast flexors and extensors produce rapid tailflips during backward swimming.

In this paper we demonstrate the different pathways taken by the slow- and fast-flexor motor neurons in the cores of the first five abdominal ganglia. The physiological and morphological identifications of these neurons have been published previously (Selverston and Remler, '72; Wine, et al., '74; Mittenthal and Wine, '78) and are not repeated here. Our results support the generality of Alt-man's hypothesis and support the behavioral and physiological observations that the slow flexors are involved in more of an animal's activities than the fast flexors (Kennedy and Takeda, '65a,b; reviewed in Wine, '84).

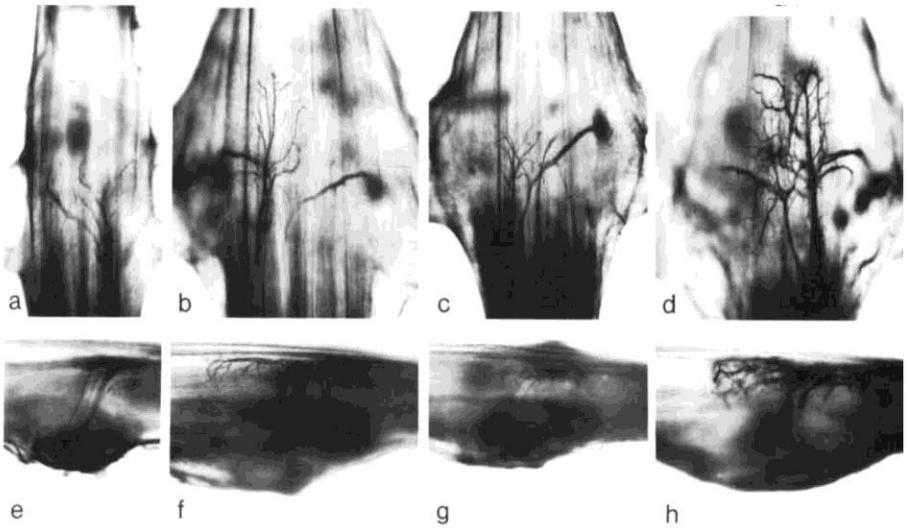
METHODS

Male and female *Pacifastacus leniusculus* (Dana) were obtained from Delta Crayfish, Rio Vista, CA; Cliff's Marina, Freeport, CA; or from California Valley Fish Co., Hood, CA. No obvious sexually dimorphic differences in the results were discerned. Crayfish were anesthetized before dissection by chilling on ice.

Backfills

For backfills (Iles and Mulloney, '71), the nerve cord was dissected out of the abdomen and pinned with cactus spines, ventral-side up, in a Sylgard-lined petri dish in cold crayfish saline (Paul and Mulloney, '85). Two contiguous Vaseline wells were made next to the nerve to be filled—either the superficial or the main branch of Nerve 3. The proximal well was filled with 0.4 M sucrose (Mulloney, '73); the nerve was placed across both Vaseline wells; and the sucrose well was covered with Vaseline. The distal well was then filled with 0.2 M cobalt chloride, and the nerve in the $CoC1_2$ solution was cut. The distal well was also covered with Vaseline. The preparation was then placed in a 7°C refrigerator and the cobalt was allowed to diffuse into the cut nerve for 1.5-2 days for fills of slow flexors and for 6-8 hours for fills of fast flexors. Longer filling times might have enabled us to fill more fast-flexor neurons per ganglion, but longer times also led to more cobalt leakage, especially from the motor giant neuron (MoG), which obscured the results. At the end of the diffusion period, the cords were rinsed in saline for 10 minutes with four to six changes of solution. The saline was replaced with 0.1 M sodium cacodylate (436 mOsM) and cobalt sulphide was precipitated by the addition of 5 drops of ammonium sulfide for every 15 ml of buffer. After 10 minutes, the reaction was stopped by rinsing the cord with saline. The nerve cord was then fixed in a solution of 10 ml of 8% glutaraldehyde and 20 ml of 0.21 M Na cacodylate on ice for 2 hours (Skinner, '85a). The nerve cord was briefly washed in buffer and transferred in steps to 70% ethanol and left there for several hours or overnight (Tyrer et al., '80). The nerve cord was then hydrated in several steps to distilled water.

Originally we used stainless-steel pins to position the nerve cords, but we found that black deposits (presumably iron sulfide) developed on the nerves and connectives near the pins during the precipitation procedure. The use of cactus spines *(Mammillaria sp.)* eliminated this problem.



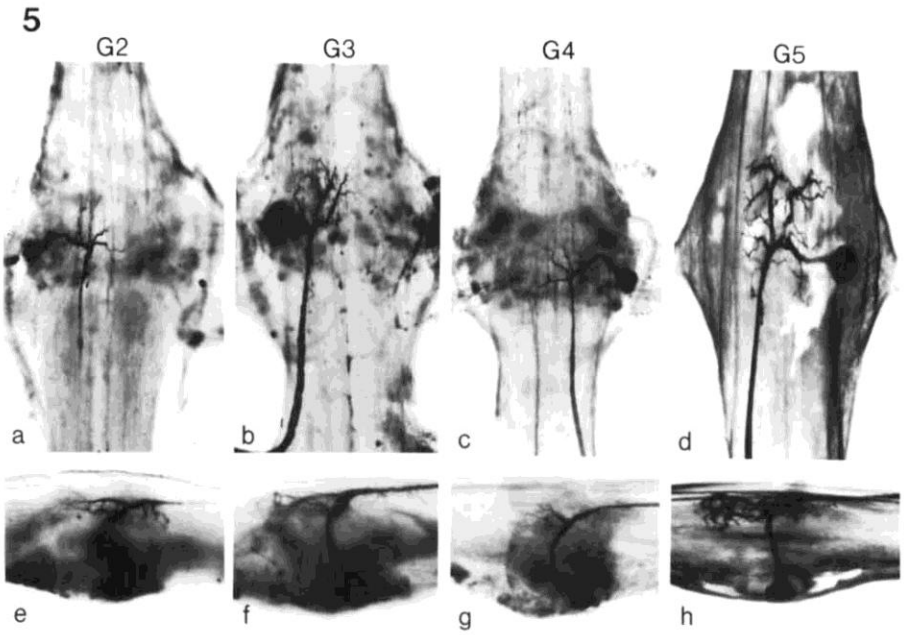


Fig. 4. ***a-d. Dorsal views of whole mounts of backfilled fast-flexor motor neurons. Anterior is up. GI has three filled neurons, G2 has four filled neurons, G3 has five filled neurons, one of which is the motor giant, and G4 contains four filled neurons. GI-G3 contain neurons that are relatively lightly filled. Neurons in G4 are well filled, and hence darker, and display more of their smaller branches. a, d, x 47, b, x 38, c, x42. e-h. Side views of above ganglia. Anterior is to the left. Note profuse branching in upper one-third of G2-G4. e, f, h, x45, g, x40.***

Fig. 5. ***a-d. Dorsal views of fast-flexor motor neurons. Anterior is up. ac. Neurons filled with HRP. x 36, x 39, x41. d. Neuron backfilled with cobalt chloride. x 59. e-h. Side views of above neurons. Anterior is to the left. x46, x34, x57, x49.***

The cords were intensified by a method originally developed for electron microscopy (Tyrer et al. '80). Cords were placed in the intensification solution that consists of 6 g sucrose, 3 g gum arabic, 0.8 g citric acid, and 0.17 g hydroquinone in 100 ml distilled water for 1.25 hours at 50°C in the dark. An equal volume of solution was simultaneously warmed in the oven. The solution on the cord was replaced with the warmed solution and 1% $AgNO_3$ was added to it in a 9:1 (intensifier: $AgNO_3$) volume ratio. The cord was replaced in the oven and then examined every 10 minutes until the filled cells began to darken. The cord was then examined every 5 minutes. Intensification was stopped by rinsing the cord in water when the filled cells were black and the cord was light brown. The cord was dehydrated in a graded ethanol series, cleared in methyl salicylate, and photographed.

Useful whole mounts were then rehydrated and postfixed in 2% osmium tetroxide in 0.1 M Na cacodylate for 4 hours on ice. Long postfixation times (> 2 hours) were used to increase the ultimate contrast of the sectioned material. After postfixation, specimens were dehydrated in an ethanol series and 100% acetone, infiltrated, and embedded in soft Spurr's ('69) resin (Leise and Mulloney, '86). No counterstain was used. Ganglia were sectioned at 20 μm and mounted in high-viscosity VH oil (Cargille, Co. or Poly-sciences, Inc.).

Fig. 6. a. Frontal section through the HN and LN of G4 (cf. Fig. 4d), with seven linking segments (circled) rising in vertical tracts G, H, and M (labeled). In the LNs, each G tract rises between longitudinal tracts VLT_i and VLT_o. The two neurites in the contralateral (right) vertical tract G will not appear in more dorsal sections. Note dendrite (arrow) in HN. ×85. b. Similar section through G3 (Fig. 5b) with one neuron (circled) filled with HRP. ×99.

Fig. 7. a. Frontal section through G4 (cf. Fig. 4d). Five well-filled neurons are represented by their linking segments in vertical tracts G, H, and M (circled and labeled). Here, in the beginning of the second (counting dorsal to ventral) tract layer, filled dendrites (arrowheads) lie within the TN between the two DITs in DC5 and DC7. Note large unfilled neurites. ×92. b. The next adjacent dorsal section. Again no large neurites are filled (filled dendrites at arrowheads). ×98. c. The next dorsal section shows dendrites in the TN (arrowheads) of the upper part of DC5–DC7 and most of the third layer of longitudinal tracts. ×112.

Fig. 8. Serial frontal sections through the upper layer of dorsal commissures through the same ganglion as Figure 7. Note the large filled neurites crossing the ganglion in the anterior half of the ring tract (part of DC2 and DC3, arrows). The four neurites (circled) visible in a give off larger branches in b. Most of the smaller branches occur in DC1, DC2, and DC3 (arrowheads), although some are very posterior in DC4 (cf. Fig. 8a., arrowhead). ×99, ×117.

Fig. 9. Serial frontal sections through G4 (same ganglion as in Fig. 8) in the first layer of longitudinal tracts. a. Some of the filled neurites are in the remains of the dorsal commissures (arrow). Filled axons are at left and run below the MG. ×103. b. Branches (arrowheads) extend across the ganglion to the contralateral MG. ×112.

Tyrer et al. ('80) suggest that intensifying solutions work best when 2-3 days old. We found old solutions to be worse than new ones because the surfaces of the nerve cords become silvered more often in older intensifiers. This occluded the view of the interior and decreased the actual intensification of the filled cells. For these reasons, we eventually used only freshly made intensifier.

Both NH_4S and rubeanic acid (dithiooxamide) were used to precipitate cobalt sulfide (Quicke and Brace, '79). Twelve drops of saturated alcoholic rubeanic acid solution added to 15 ml of buffer yielded a reddish-brown precipitate in filled neurons after 10-15 minutes. The use of rubeanic acid was suggested for studies in which two populations of neurons are filled with different markers (i.e., nickel and cobalt or copper chloride), because rubeanic acid forms unique colored precipitates with each ion (Sakai and Yamaguchi, '83). In sections through ganglia processed with rubeanic acid, the contrast between the filled neurons and background was quite low. Thus, we ultimately abandoned this technique.

The intensification procedure we used (Tyrer et al., '80) is more sensitive to the quality of the fill than our earlier method (Tyrer and Bell, '74). Dark, well-filled cells intensify to a deep black, whereas lightly filled neurons intensify to a red-brown color. This allowed us to judge the quality of the fills. We used this characteristic to eliminate poorly filled neurons from our final results. Data presented below are selected from a total of 48 well-filled ganglia. Care was taken to include only descriptions of cleanly filled cells. If extensive leakage occurred that obscured the data, the preparation was not used. We found that lightly filled cells did not confound the data. Rather, descriptions of such fills underestimate the amount of dendritic branching but did not cause us erroneously to describe filled neurites.

In the most ventral cobalt sections (Fig. 16a—c) it is difficult to distinguish between the horseshoe neuropil (HN) and lateral neuropils (LNs) by their cytological appearances. Distinctions made here are based on sections of ganglia prepared by the osmium-ethyl gallate technique (Wigglesworth, '57), in which these two neuropils stain differentially (Skinner, '85a; Leise and Mulloney, '86) as a result of their ultrastructural differences (Skinner, '85b).

Intracellular fills

For intracellular fills, nerve cords were dissected as described above and pinned out ventral-side up, and ganglia 2-5 (G2—G5) were desheathed. The cord was twisted 180° between G1 and G2 and between G5 and G6 to allow access to the giant fibers. Pin electrodes (Mulloney and Selverston, '74) were used to record from or to stimulate the giant fibers and the three pairs of nerves that emerge from a single ganglion. Omega-dot, glass capillary microelectrodes with tip resistances between 20 and 40 MΩ (in 2.5 M KC1) were used to impale neuronal somata. After being filled with 2% horseradish peroxidase (HRP, Sigma type VI) in 0.2 M Tris base and 0.5 M KCl, pH 7.4 (Watson and Burrows, '83), such electrodes had tip resistances between 150 and 200 MO. Our Getting Microelectrode Amplifiers were modified to allow us to balance the bridge on these high-resistance electrodes. Fast-flexor motor neurons were identified by the moccurrence of attenuated action potentials in the somata and spikes in the third nerves in response to giant fiber stimulation. Antidromic stimulation of the third nerves also elicits attenuated action potentials in the somata (Selverston and Remler, '72; Wine et al., '74; Mittenthal and Wine, '78).

Motor neurons were filled with HRP by passing 2-5 nA of current in 500-msec pulses at 1 Hz for 1 hour. Currents were measured with a virtual-ground current monitor. Larger amounts of current would not pass through these electrodes. After an experiment, the nerve cord was placed in saline overnight at 7°C. It was then fixed as described above for 2 hours, washed in isosmotic (436 mOsM) 0.1 M Na cacodylate buffer, and incubated for 3 hours, on a rotator, at room temperature in 0.4 mg/ml diaminobenzidine (DAB) in the same Na cacodylate buffer in the dark. Then, 2 drops of 3% H_20_2 was added and the preparation was allowed to react for 1 hour, again in the dark and on the rotator. The specimen was rinsed in distilled water, dehydrated in ethanol, cleared, drawn, and photographed. Post-fixation and subsequent treatment were as described above.

General considerations

We found that the dendritic domains of the filled neurons cannot be completely understood from whole mounts. The very narrow branches (~1 μm in diameter) are difficult or impossible to discern in whole mounts, so the number and extent of dendritic branches are underestimated until one examines the resultant sections.
All photographs were taken with 120 size Pan X or Tech Pan 6415 film. Sections and HRP whole mounts were photographed with a blue filter to enhance contrast (Mees and Eastman, '74).

In single frontal sections it is often difficult to identify absolutely the end of one commissure or longitudinal tract from the beginning of the adjacent one, e.g., dorsal commissure (DC) 1 from DC2, or dorsal intermediate tract (DIT) from dorsal lateral tract (DLT). The designated tract and commissure names that are labeled on the micrographs are made from studies of serial sections. However, there are instances where adjacent structures touch. For example, the posterior edge of DC3 abuts the anterior edge of DC4. Also, the dorsal edge of DC7 is continuous with the ventral edges of DC3 and DC4. Our labels should not be regarded as dogma; they are included to aid the reader's understanding.

REVIEW OF GANGLIONIC STRUCTURE AND TERMINOLOGY

The internal anatomy of the fourth abdominal ganglion in *Procambarus clarkii* has been described previously (Skinner, '85a,b). Each abdominal ganglion contains a ventral rim of neuronal somata, four layers of axonal tracts (many of the axons run the entire length of the abdominal nerve cord), three layers of commissures (Fig. 2), and four different types of neuropil (Skinner, '85b). The anterior ventral commissure (AVC) is out of the plane of Figure 2. Tract neuropil (TN) lies between and within all of the tracts and commissures and is not included in Figures 2 or 3. All of these structures occur in *Pac. leniusculus* (Fig. 2) with a few minor exceptions. We found no posterior ventral commissure nor its overlying midline neuropil. In *Pac. leniusculus* but not in *Proc clarkii* the ventral regions of the lateral (LN) and horseshoe (HN) neuropils touch (Fig. 3).

Crayfish motor neurons are unipolar neurons whose somata are electrically remote from synaptic transmission (Takeda and Kennedy, '64). A relatively thin, unbranched neurite, or linking segment, arises from the soma. Within the ganglionic core this neurite expands and supports many dendritic branches. This branched portion, or integrative segment, integrates excitatory and inhibitory synaptic input that give rise to the action potential at the still-more-distal spike-generating locus (Cohen, '70). We will use these terms in our descriptions of the neuronal routes and sometimes refer to dendrites by their branch order—primary or secondary.

Skinner's descriptions of ganglionic structures are an advance over Kendig's ('67) study with the exception of the identification of the vertical tracts. By her use of the osmium-ethyl gallate staining technique, which preserves more ganglionic structure than the silver impregnations used by Kendig (Leise and Mulloney, '86), Skinner was able to distinguish several types of neuropil from both the longitudinal and vertical tracts within the core of the ganglion. Skinner retained the insect nomenclature (Pipa et al., '59; Gregory, '74; Tyrer and Gregory, '82) to emphasize the morphological similarities between the crayfish ganglia and those of insects. However, until the functions of the various tracts and neuropils are compared, it remains uncertain that these structures are truly homologous.

We used Skinner's ('85a) nomenclature, but a few of our terms differ from hers and from those used in other crayfish literature. Technically, "nerves" emerge or enter ganglia and once inside the ganglia divide into bundles of axons or "roots" (Gregory, '74; Bloom and Fawcett, '75). This terminology is used consistently by neuroanatomists studying insects (Gregory, '74; Ernst et al., '77; Burrows, '80; Tyrer and Gregory, '82; Siegler, '84) and vertebrates, but is used incorrectly in much of the crustacean literature (Selverston and Remler, '72; Wine et al., '74; Mulloney and Selverston, '74; Kramer et al., '81; Wine, '84; Kramer and Krasne, '84; Skinner, '85a,b; Leise and Mulloney, '86). The term "nerve root" is often used when "nerve" is meant. Adding to the confusion, Skinner ('85a) refers to nerve roots as "root bases." We prefer to use the terms "nerve" and "root" in accordance with their original definitions.

The second commissure layer in crayfish ganglia lies just below the second layer of longitudinal tracts (Fig. 1). The second commissure layer in insect ganglia lies in an analogous position. Skinner refers to this commissure layer as a "ventral" commissure while Tyrer and Gregory (Gregory, '74; Tyrer and Gregory, '82) call it a "dorsal" commissure. To simplify comparisons of these ganglia, we return to Tyrer and Gregory's nomenclature and call the second commissure layer "dorsal." Our numbering scheme for commissures (1-4 in the top layer, 5-7 in the lower layer) differs from theirs because there are more dorsal commissures in crayfish and because the commissures are larger than the insect ones and occur in different positions.

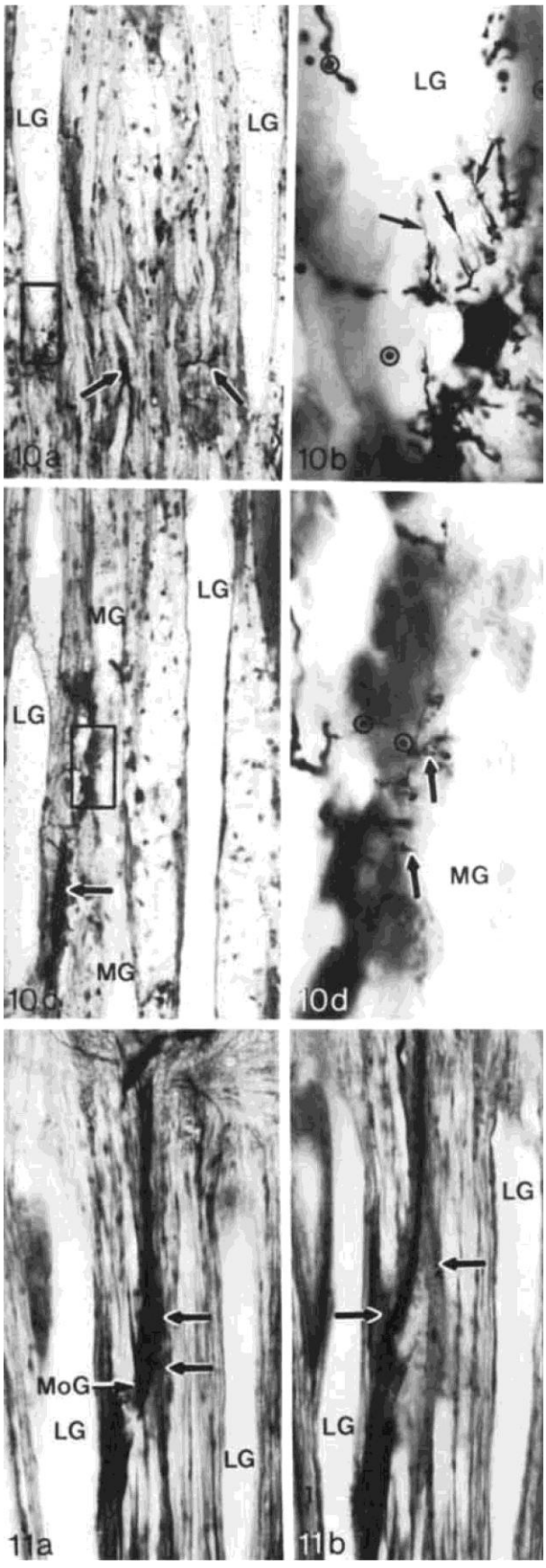


Fig. 10. Frontal sections through the first longitudinal tract layer, HRP-filled fast-flexor motor neuron (cf. Fig. 5b,f). a. Area of contact (rectangle) between a lateral giant axon (LG) and a fast-flexor motor neuron (arrows) enlarged in b. c. Area of contact (rectangle) between a medial giant axon (MG) and a fast-flexor motor neuron (arrows) enlarged in d. (Circled dots in b and d are encysted parasites, arrows point to dendrites.) a ×70, b ×517, c ×88, d ×506.

Fig. 11. Serial frontal sections through a ganglion in which the motor giant neuron (MoG) has been backfilled with $CoCl_2$. Dendrites (arrows) in a and b branch in the MDT. a,b ×73.

RESULTS

Main paths of fast-flexor motor neurons

Several characteristics of the morphology of the fast-flexor motor neurons can be observed from whole mounts. The somata of the fast-flexor motor neurons, ranging in diameter from 20 to 85 gm in *Proc. clarkii* (Mittenthal

and Wine, '78), are among the largest in the ganglion. The somata occur in three clusters: an anterior group with axons in the contralateral third nerve of the adjacent anterior ganglion; a medial group with contralateral axons; and a posterior group with ipsilateral axons (Mittenthal and Wine, '78). Between seven and 11 fast-flexor motor neurons occur in each of ganglia 1-5 in *Proc. clarkii;* the more anterior ganglia contain more neurons. All three clusters are represented in each ganglion except G5, which lacks the anterior group.

Fig. 12. Frontal sections through three different ganglia with fast-flexor motor neurons filled with cobalt. Sections are through the dorsal commissures, DC1–DC4. Filled neurites cross the ganglion orthogonally in DC3 and DC4. a. Four filled neurons in G3, including the MoG (arrowhead). Large branches cross the ganglion in DC3 and DC4 (arrows). ×86. b, c. Serial sections of two filled neurons in another G3. The more lightly filled cell crosses the ganglion in DC3 (arrows) in b. ×78. c. The most anterior neurite crosses in DC2 (arrow). ×110. d. G1, four filled cells. Cells branch in the dorsal part of DC3 and in DC4 (arrows). ×97.

Fig. 13. ***Comparable frontal sections through the second longitudinal tract layer in two ganglia that contain different fast flexors filled with HRP. Dendritic branches occur in both M tracts (circled). Main neurites at arrowheads. a. G3 (cf. Fig. 5b). Note branches in anterior TN (arrows). x 138. b. G2 (cf. Fig. 5a). Note lack of dendritic branches in anterior TN. × 138.***

Fig. 14. ***Two frontal sections through the main part of the first commissure layer (DC1—DC4) from ganglia that contain two different fast flexors filled with HRP. Note relative differences in dendritic branching in***

these two sections (arrows). Main neurites at arrowheads. a. G3 (cf. Fig. 5b). × 138. b. G4 (cf. Fig. 5c). × 138.

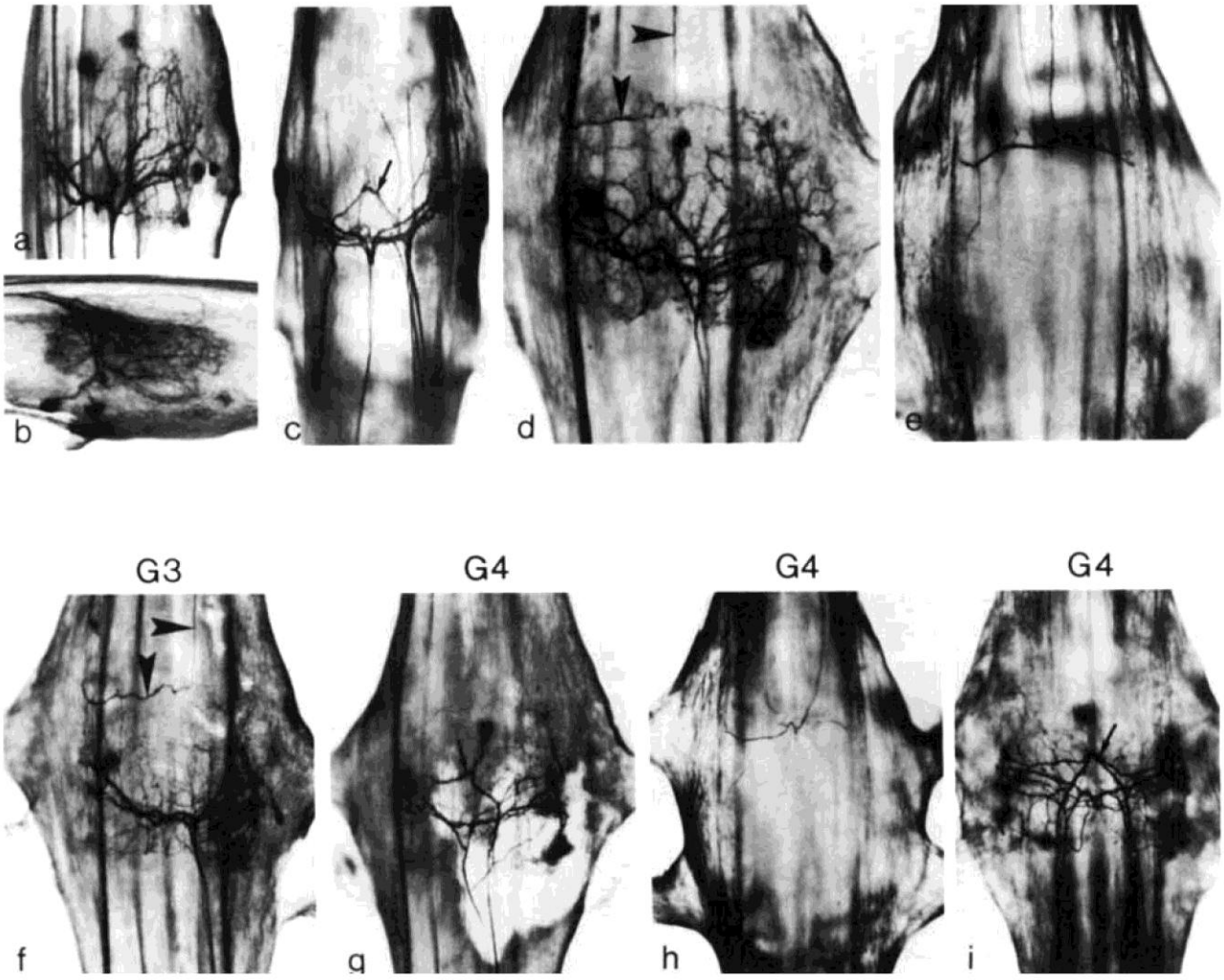


Fig. 15. Whole mounts of G1–G4 with slow-flexor motor neurons backfilled with cobalt chloride. All dorsal views unless otherwise stated. Anterior is up. a. G1, five cells filled. ×47. b. G1, side view of same ganglion as in a and anterior is to the right. Note dendrites extend across entire core of ganglion (anterior to posterior) and cover most of the ganglion, from dorsal to ventral layer of somata. ×45. c. G1, five cells filled. ×43. d. G2 six cells filled. Arrowheads mark neuron f1 that exits in the adjacent anterior Nerve 3. ×56. e. G2, two cells filled from the next anterior Nerve 3. ×60. f. G3, five cells filled, note lack of central cell (f3) and its inverted V-shaped projection visible in c, d, g, i (arrows). Arrowheads mark the f1 neurons from the next anterior Nerve 3. ×47. g. G4, five cells filled ×47. h. One cell filled from adjacent anterior Nerve 3. ×49. i. Bilateral backfill, G4. Nine filled cells. ×54.

The linking segments, or main neurites (Wine et al., '74; Mittenthal and Wine, '78), between the somata and presumed integrating segments (Cohen, '70) are 5-16 gm in diameter, usually lack branches (Figs. 4 and 5 side views), and are between 300 and 400 Am long. Most of the dendritic arborizations occur in the dorsal third of the ganglion (Figs. 4 and 5 side views). The linking segments rise through the ganglia in one of several vertical tracts (Fig. 4a—d), depending upon the location of the somata. Each integrating segment gives off several primary branches that carry most of the finer dendrites, but the specific branching pattern is an exclusive characteristic of each neuron. These fast-flexor neurons branch mostly on the ipsilateral side of the ganglion, but many also send dendrites to the contralateral hemiganglion. All of the axons exit the core of the ganglion very dorsally.

Domains of fast-flexor dendrites

The locations of the dendritic branches in the preparation with the greatest number of well-filled neurons (Fig. 4d,h) are described below. Where necessary, comparisons of similar regions from other preparations will be made to illustrate additional points.

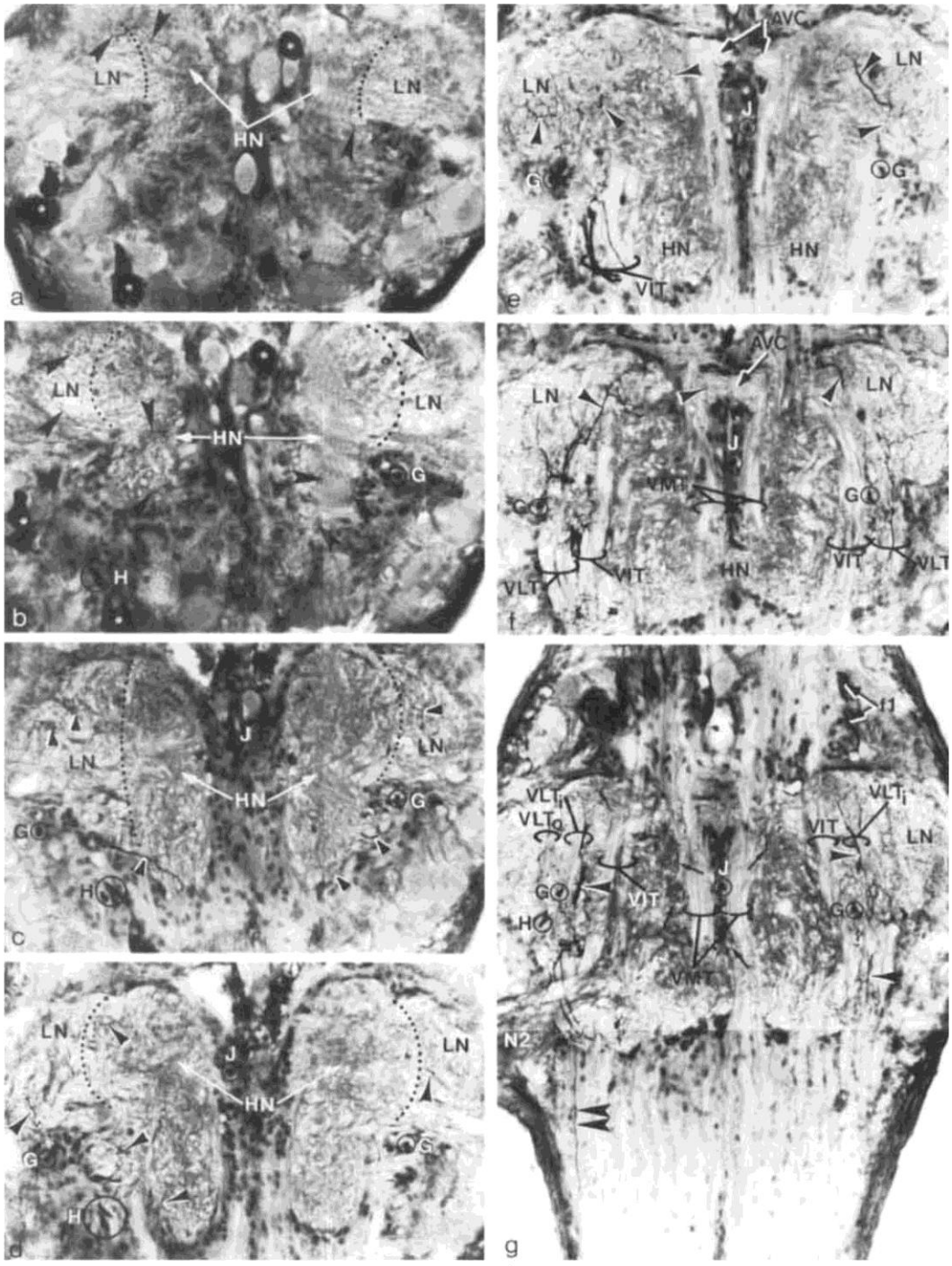


Figure 16

Fig. 16. ***a,b. Serial sections through the LNs and the ventral arms of the HN in G2 (cf. Fig. 15(1). Main neurites are circled. Approximate lateral extent of the HN is marked by the dotted lines. Three of the filled somata (asterisks) are included. Dendrites are at arrowheads. × 100, ×109. c, d. Serial sections, note positions of dendrites in HN and in LNs (arrowheads). Five neurites occur in two G, one H, and the J tracts (labeled). x 101. e,f. Serial sections. In addition to dendrites (arrowheads) in both LNs they occur in the anterior portion of the HN (more ipsilaterally) and in the AVC. Two neurites in H now occur as longitudinal neurites in the ipsilateral (left) VIT. In f branches cross from the LNs to the HN. The remaining three neurites (one in each G and one in J) are still visible as unbranched neurites. x96. g. Section through the curve of the HN and LNs. VLT_i and VLT_0 occur in the left LN. Neurites in G and H rise between these two tracts. Note longitudinal branches in the VLTs (arrowheads). Dendrites occur in the medial areas of HN (arrows) and in both LNs. Also note dendrites crossing the root of the left Nerve 2 and the branch (double arrowhead) that descends the connective in the VLTs. The soma and linking segment of fl are also visible in this section. × 88.***

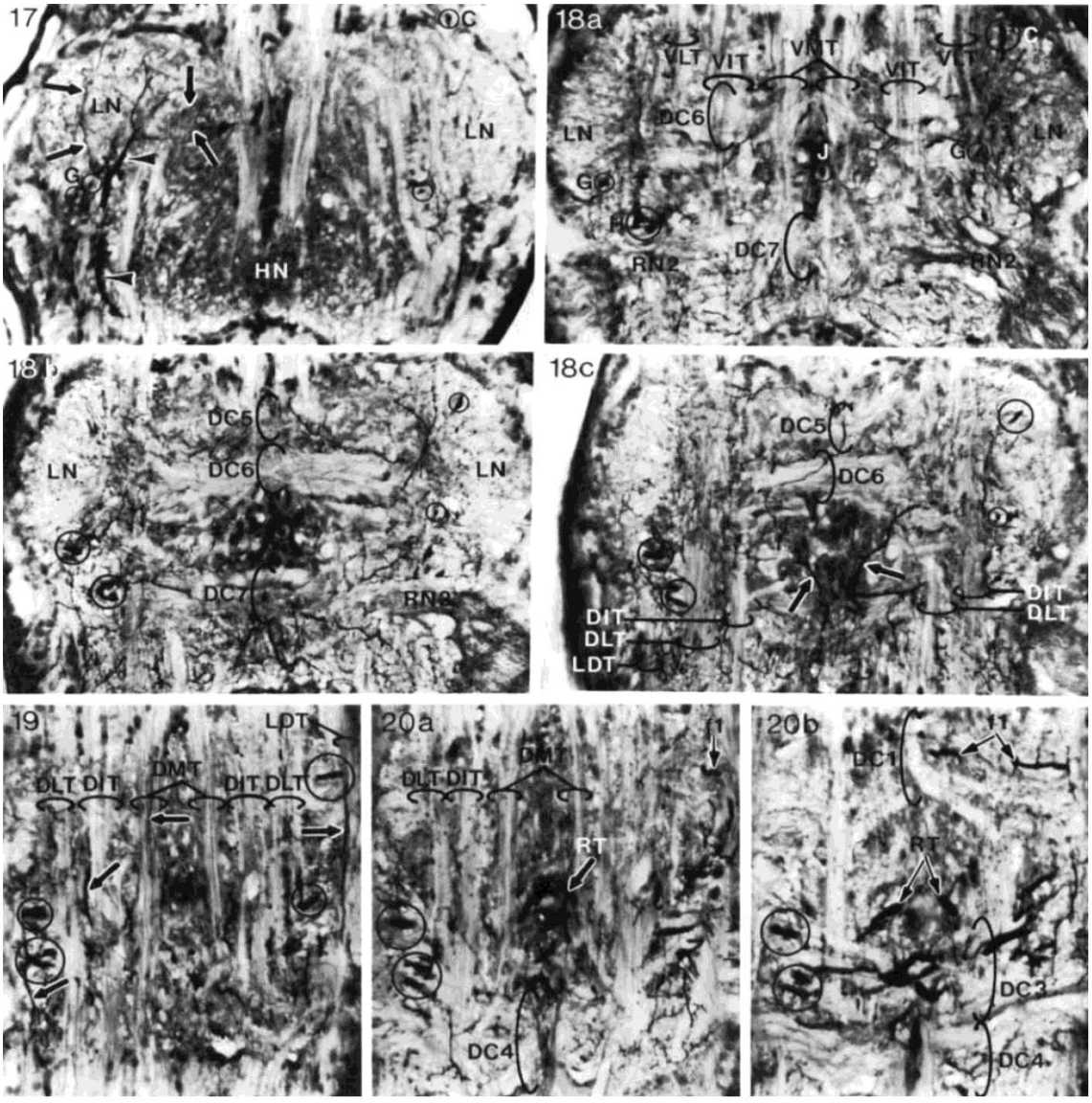


Fig. 17. G3, five filled slow flexors. Neither f3 was filled and no filled neurite occurs in J. One neurite in H has sent a primary branch into the VLT_i (arrowheads). The other four neurites are circled. The primary neurite in the VLT_i also sends secondary branches (arrows) into the LN and anterior HN. ×92.

Fig. 18. Three serial sections of G2 (cf. Fig. 15d). Six filled slow flexors. Main neurites are circled. a. The third longitudinal tract layer and part of second commissure layer. ×100. b. Second commissure layer. ×100. c. Dorsal part of second commissure layer and part of the second longitudinal tract layer. In this section three ipsilateral neurites have started to cross the ganglion. f3 has given off several large branches (arrows) that lie between the two DITs. ×100.

Fig. 19. G3 (cf. Fig. 15f). Five filled slow flexors. The integrative segments of f1, the three ipsilateral, and one contralateral neuron are circled. Note longitudinal branches in LDT, DMT, DIT (arrows). ×96.

Fig. 20. Serial sections through G2, six filled slow flexors (cf. Fig. 15d). Three ipsilateral neurites are circled. a. Second longitudinal tract layer and beginning of DC1–DC4. Note filled neurites from f3 in DC2 in the anterior ring tract (RT). ×109. b. First commissure layer. f3 branches (RT) continue in DC2 and DC3. Anterior neurite is from f1. ×109.

In G4 (Fig. 4d), five neurons were well filled and two other cells were filled only in their somata and linking segments. Four vertical tracts contain the seven filled linking segments: two lie in each of the G tracts, one in the ipsilateral H tract, and two rise in the M tract (Fig. 6). Some of the linking segments give off a few fine branches and contribute to the tract neuropil of the vertical tracts. A few dendrites descend into the horseshoe neuropil (HN) (Fig. 6a), but generally, the fast-flexor motor neurons do not send branches into the HN and lateral neuropils (LNs). This is confirmed in sections from neurons filled with HRP, where we are more certain that each neuron is completely filled (Fig. 6b).

A few sparse branches occur in the tract neuropil (TN) of the third layer (counting dorsal to ventral) of longitudinal tracts (not illustrated): between the ventral intermediate tracts (VITs) and dorsal lateral tracts (DLTs) at the edge of the ganglion. Branches also occur in the TN above the HN, between the ventral medial

(VMT) and intermediate WIT) tracts on each side. The ipsilateral linking segments in vertical tract G give off a few fine branches in the LN.

The branching of the fast-flexor motor neurons becomes more profuse in the dorsal commissures and upper two layers of longitudinal tracts. The TN of the central part of the ganglion, between the two DITs and in DC5, DC6, and DC7 contains many fine, filled dendrites (Fig. 7). However, none of the larger neurites in these commissures is part of fast-flexor motor neurons. The branching becomes even more abundant in the more dorsal sections. Vertical tracts G and H ascend through the LN and then between the DLT and LDT on each side. Midway into the layer of DC5—DC7, (Fig. 7b) the three ipsilateral linking segments in G and H tracts begin to branch. Most of the fine branches from the primary neurites lie between the two DITs (Fig. 7b) but some do lie in the TN between the DITs and DLTs, again mostly ipsilaterally. None of the large longitudinal neurites in the second layer of tracts (dorsal median tracts =DMTs, DITs, DLTs) are fast-flexor neurites (Fig. 7c).

In the dorsal layer of commissures, DC1—DC4, the number of finer branches increases, mostly centrally. There are also large neurites filled in DC2 and DC3 (Fig. 8a,b) that are part of the ring tract (RT). Many fine branches occur in the TN of these commissures and span the core of the ganglion, from its anterior to posterior edges (Fig. 8). The large linking segments ascending in tracts G, H, and M branch in this region of the ganglion (Fig. 8a,b), which suggests that this is the beginning of the integrative segments of these neurons. Although most of the primary branches lie in the plane of the upper level of commissures, the secondary and tertiary branches descend into the longitudinal tracts below (Fig. 7) and ascend into the dorsal-most layer of longitudinal tracts (Fig. 9). In this dorsal layer, fine branches span the ganglion, lying in the median dorsal tracts (MDTs) and lateral dorsal tracts (LDTs) below and adjacent to both medial giant axons (MGs). Points of contact between these dendrites and the giant fibers are better viewed in HRP intracellular fills (Fig. 10). The axons exit from the ganglion in the LDT and MDT, passing under the MG (Figs. 9 and 11) and over the lateral giant axon (LG).

Additional fast-flexor pathways

Other ganglia contained filled fast-flexor motor neurons that were not filled in the ganglia described above. In two ganglia, the motor giant neuron (MoG) was visible (Fig. 11). From its contralateral soma, the MoG linking segment rises up through the center of the ganglion caudal to tract K, lateral and adjacent to both the VMT and DMT (Skinner, '85a). It crosses to the ipsilateral side of the ganglion in the first commissure layer, exits the ganglion in the MDT and branches in the MDT near the midline. With the other fast-flexor motor neurons, it passes under the MG, over the LG, and enters Nerve 3.

Several large primary branches in addition to those previously described in the ring tract (RT) cross the ganglion in commissures DC4, DC3, and DC2 (Fig. 12c). Thus, many of the large neurites in DC3 and a few of the anterior ones in DC4 belong to fast-flexor motor neurons.

The amount of dendritic branching in the first and second commissure layers and the first and second longitudinal tract layers varies in different neurons. For example, in G4 with five filled neurons (Fig. 4d,h), we found that the finer dendritic branches in the second longitudinal tract layer spanned the central part of the ganglion from the anterior to the posterior edges of the core (Fig. 7c). The total branching patterns we report are composites from neurons that branch in different parts of the ganglion (Figs. 13, 14). The differences in branching patterns of the large neurites are obvious from whole mounts (Fig. 5), but the differences in the placement of the narrower dendrites are best seen in sections (Figs. 13, 14).

General features of slow-flexor motor neurons

Six pairs of slow-flexor motor neurons have been identified in each abdominal ganglion of *Prop clarkii* (Wine et al., '74). The number and positions of the slow-flexor somata and the general branching patterns that we observed in *Pac. leniusculus* resemble those described by Wine et al. We adopt their nomenclature here.

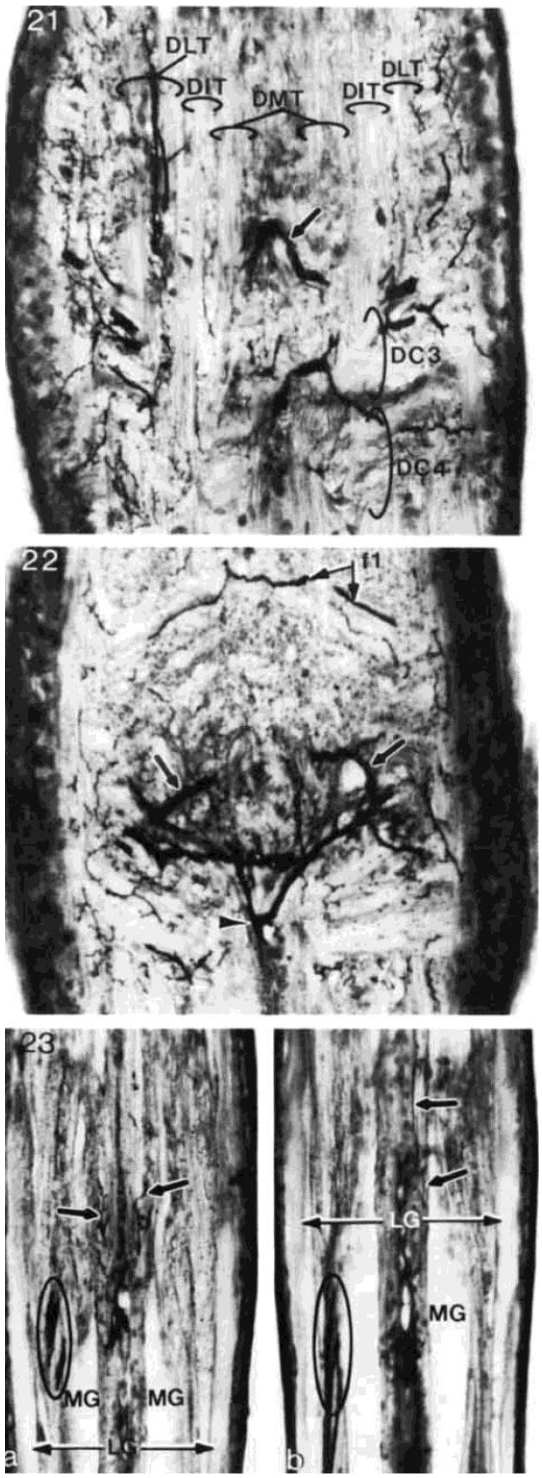


Fig. 21. G1, five filled slow flexors (cf. Fig. 15a,b). Section is through second tract and first commissure layers. f3 (arrows) bifurcates in the RT. Note longitudinal branches in the left DLT. ×136.

Fig. 22. G4, five filled slow flexors (cf. Fig. 15g). Section through DC1–DC4. Both branches from f3 cross the ganglion from DC2 to DC3 (arrows). The axon from the ipsilateral branch exits the core at the arrowhead. The anterior neuron, f1, crosses the ganglion in DC1. ×118.

Fig. 23. Serial sections through G2 (cf. Fig. 15d). Six filled slow flexors. MDTs contain most of the dendrites at this level (arrows). Five axons exit the ganglion at the left (in elipse). a is ventral to b. ×86.

At most, we filled six cells from the superficial branch of any Nerve 3 (G1-G4) (Fig. 15). The major neurites of the slow flexors travel in distinctive pathways visible in the whole mounts (Fig. 15). Most of the larger branches from these neurons lie dorsally within the ganglion (Fig. 15), and their large neurites cross the ganglion in one of the posterior dorsal commissures. Five of these neurons, three ipsilateral and two contralateral, have somata and dendritic trees in that segment's ganglion. The sixth cell, fl (Wine et al., '74), has its soma and branches in the next posterior ganglion. The neurites of fl also branch in characteristic areas—two branches cross the ganglion very anteriorly (Fig. 15e,h) and smaller branches descend through the longitudinal tracts into the ganglionic core. The midline cell, f3, has a contralateral soma and neurites that made an inverted "V" shape (Fig. 15a,c,d,g,i) that is absent when this cell is not filled (Fig. 15f). This inverted "V" of f3 is deeper than the major commissural crossings mentioned above.

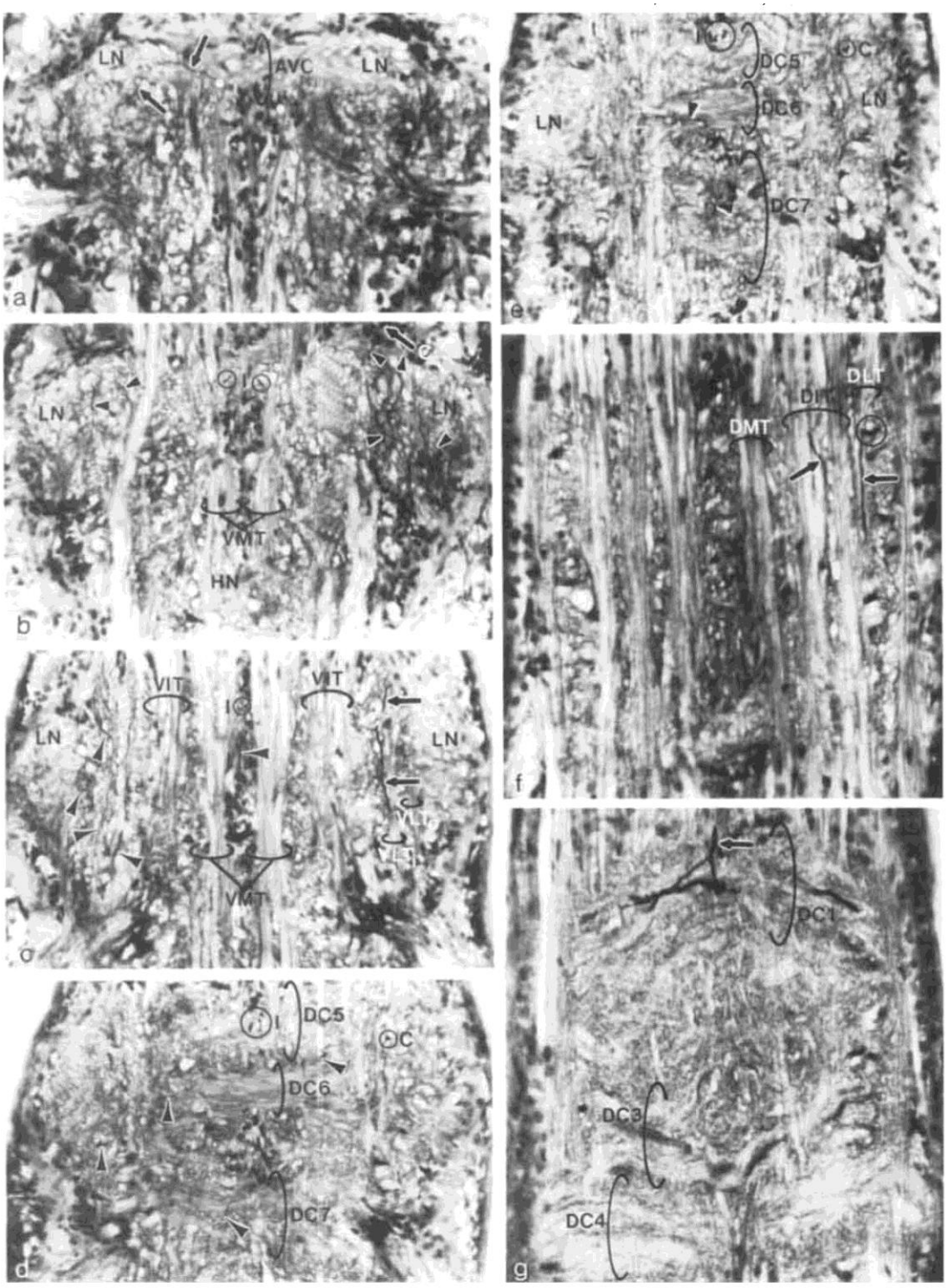


Figure 24

Fig. 24. ***Sections from a bilateral fill (one side incomplete at lower levels) of the two fls that have axons in the adjacent anterior N3 (cf. Fig. 15e). a. A very ventral section, with filled dendrites (arrows) in the AVC. x 113. b. Filled dendrites in the LNs (arrowheads). Two major secondary branches descend through the vertical I tract (circles). The linking segment rises in vertical tract C, just at the edge of the section. x 110. c. Secondary branch in VLT, (arrows) of third tract layer. Dendrites are at arrowheads and branches in I are circled. x 120. d,e. Serial sections through DC5-DC7. Very few filled dendrites (arrowheads). The two branches in I and the integrative section in C are circled. x 104, x 108. f. Second longitudinal tract layer. The integrative segment is circled. Note two primary branches in DIT and DLT (arrows). x143. g. DC1, DC3, and DC4. Axon exits at midline (arrow). Primary branch crosses the ganglion in DC1 (cf. Fig. 15e). x143.***

Unlike the fast flexors, which branch only in the dorsal part of the ganglion, the slow flexors send fine branches into a large portion of the ganglionic core. From the whole mounts, it is clear that several slow flexors send longitudinal secondary branches into the ganglion, and many tertiary branches arise from these neurites. Their dendritic trees cover the core from its anterior to posterior edges and descend almost to the ventral layer of somata (Fig. 15b).

The main neurites of all but one of the slow flexors, each from 4 to 12 μm in diameter, give off many branches as they rise through the ganglion. The exception is f3, whose main neurite remains relatively unbranched until it bifurcates dorsally (Fig. 15d). The linking segments of f2, f4, f5, and f6 are between 150 and 250 μm long, about two thirds as long as that of f3.

Domains of slow-flexor dendrites

Dendrites from slow flexors occur much more ventrally and cover a greater proportion of the ganglion than do those from fast flexors. All of the dendrites in the most ventral part of the ganglion descend from above. Narrow processes, 0.5-2 μm in diameter, occur in the most ventral regions of the LNs, in the ventral parts of the arms of the HN where the LNs and HN are still joined (Fig. 16a-d), and posteriorly in the HN (Fig. 16c,d). Some of these branches are bilaterally symmetrical. In the slightly more dorsal sections, in which the posterior curve of the HN begins to appear, there are generally more ipsilateral dendrites (Fig. 16d-f).

The five main neurites have numerous branches in the HN and LNs at their midlevels and rise in four tracts: one in each G, two in the contralateral H tract, and one in J (Fig. 16d). The most ventral of the primary branches arise from the two neurites in tract H (Fig. 16e,f) and extend longitudinally and anteriorly in the ipsilateral inner $VLT_{(i)}$ (Figs. 16e-g, 17). These ventral primary branches send secondaries into the LN and anterior part of the HN. Fine dendrites also occur in the contralateral areas (Figs. 16g, 17). Some branches occur medially in the HN, near vertical tract M and on each side near the ventral median tracts (VMTs), but most of the HN lacks processes of the slow flexors. In each complete fill and in fills lacking either f3 (Fig. 150 or the neuron in the ipsilateral G tract (Fig. 15g), several processes cross the root of the ipsilateral Nerve 2 and branch very posteriorly in the ganglion (Fig. 16g). One long process also extends caudally for a short distance into the connective in the ipsilateral VLT. Branches also occur anteriorly in the AMN above the AVC (Fig. 16g).

Above the large branches in the VLT_i, the integrative segments continue to ascend in their original tracts (Fig. 18). All of the ipsilateral neurons send branches, 7-10 Am in diameter, to the opposite side of the ganglion in the first commissure layer. Many fine dendrites descend from these branches into the second commissure layer and into the second and third longitudinal tract layers (Fig. 18). Branches descend contralaterally to the second tract layer (Fig. 18a) in vertical tract G or H.

There is profuse branching in the TN posterior to the roots of Nerve 2 in the second and third tract layers and in the second commissure layer (Fig. 18a-c). The LNs also continue to receive dendrites, mostly on their medial and posterior edges (Fig. 18a-c).

The central part of the ganglion in the third tract layer also has many dendrites. These occur mostly in the TN between the major tracts, but also within the tracts themselves (Fig. 18a). No large axons in these longitudinal tracts are parts of slow-flexor neurons. No axons in the second commissure layer are filled (Fig. 18b,c) although the TN between DC5 and DC6 and between DC6 and DC7 contains filled dendrites. Filled dendrites also lie within the commissures (DC5 and DC7) themselves (Fig. 18b). The TN within the DITs and DLTs of the second tract layer contains many filled branches. There may be more filled branches ipsilaterally but generally branches are dispersed equally in the two hemiganglia (Fig. 18c).

The midline cell f3 sends out several primary branches, approximately 5 Am in diameter, in the contralateral central part of the core, between the two DITs. These branches send fine dendrites into adjacent TNs and are below the "V"-shaped branches that are apparent in whole mounts (Fig. 15d,g,i). More filled dendrites also appear in the central part of the core than did below (Fig. 18b,c). The "V"-shaped branching of this cell occurs

in DC2 as part of the RT (Figs. 19-22). These two branches cross the ganglionic core orthogonally into DC3. This neuron's axon arises from the ipsilateral branch and exits the ganglion at the midline (Fig. 22).

The three ipsilateral flexors and one contralateral slow flexor send primary branches 7-10 μm in diameter across the ganglion in DC3 and the anterior part of DC4 (Figs. 19, 20). From these branches, narrower secondary branches extend anteriorly and posteriorly through the longitudinal tracts (Figs. 19b, 20, 21). Most of the dendrites in this first commissure layer are posterior and lateral. Dendrites from fl permeate the TN in DC1, but most of the fine branches from the remaining five slow flexors arborize in the posterior part of DC2 and in DC3 and DC4 (Figs. 20, 21). Laterally, the dendrites extend across the entire width of the ganglion and reach anteriorly (Fig. 21) into the region of DC1.

The dendritic trees of the slow flexors do not extend above DC1-DC4 except for a few branches in the MDTs (Fig. 23a,b). Most, but not all, of the dendrites are below the giant axons (Fig. 23b). We found no points of contact between the slow flexors and the giant axons. Like fast flexors, the slow flexors pass under the MG and over the LG before exiting the nerve cord.

Path of the anterior slow-flexor motor neurons

G2-G5 each have two slow-flexor motor neurons (fls) whose axons exit the nerve cord in the adjacent anterior Nerve 3. The fls have relatively unbranched linking segments (Figs. 16g, 17) that ascend in the vertical tract C near the anterior edge of the LN and move into the LN just above the AVC. The fls send fine dendritic processes into the AVC (Fig. 24a) and into the anterior medial edges of the HN near the VMTs. These dendrites descend from branches that cross the ganglion more dorsally. Some dendrites also branch in the LNs (Fig. 24b) and within the TN of the VMTs and the central vertical tracts. One primary branch also runs in the VLT_i (Fig. 24c) and sends dendrites into the posterior part of the LN. Most of the dendrites in the LNs lie between the VIT and outer $VLT_{(0)}$ (Fig. 24c). Most of the HN however, contains no processes from fl.

The integrative segment continues to ascend in tract C to DC1 (Fig. 19), where it sends a major primary branch about 7 μm in diameter across the ganglion to the contralateral side. The axon and several major side branches arise from this branch. The secondary side branches are longitudinal processes that extend into the TN of the DITs and DLTs in the second longitudinal tract layer (Figs. 19, 240. These processes only extend about halfway across the ganglionic core. Two of these side branches descend vertically into the anterior midline neuropil (AMN), in the medial of the three vertical I tracts (Figs. 24b-e, 25). These two neurites give off side branches that extend in the TN of the central vertical tracts, J and M, in the third longitudinal tract level (Fig. 24c).

Aside from the filled branches in DC1 and VLT_i, no other larger neurites are filled in any of the longitudinal tracts or commissures. A few dendrites occur in DC5-DC7 (Fig. 24d,e), but no more branches appear in the LNs. A scattered few appear in the anterior TN of the DITs. A thin branch about 1μm in diameter diagonally traverses the ganglion in DC6 and DC7.

The fl axons exit bilaterally, one on each side of the midline, but their routes are not usually symmetrical. One extends along the midline to the anterior Nerve 3 and the other crosses under the MG immediately after exiting the ganglionic core and then runs laterally to its Nerve 3. This axonal asymmetry was seen in several fills of G2. We do not know if these axonal pathways are normally variable, or if this is peculiar to G2. We saw no filled dendrites from these neurons in the first tract layer and so we assume all the dendrites in the MDTs are from the other five slow flexors.

DISCUSSION

In *Pac. leniusculus* the positions of the somata vary between ganglia in one nerve cord and between corresponding ganglia in different nerve cords, as they do in *Proc. clarkii* (Wine et al., '74; Mittenthal and Wine, '78). In contrast, the pathways taken by the main neurites, the flexor linking and integrative segments, and by many of the larger primary and secondary branches do not vary. Even cells with seemingly aberrant branching patterns (compare Fig. 15a and c), prove, upon being sectioned, to lie in their normal tracts. This is

not unexpected, as identified neurons are routinely found in specific tracts and neuropils within insect ganglia (Watkins et al., '85; Watson et al., '85; Burrows and Watson, '86; Pfliiger et al., '86).

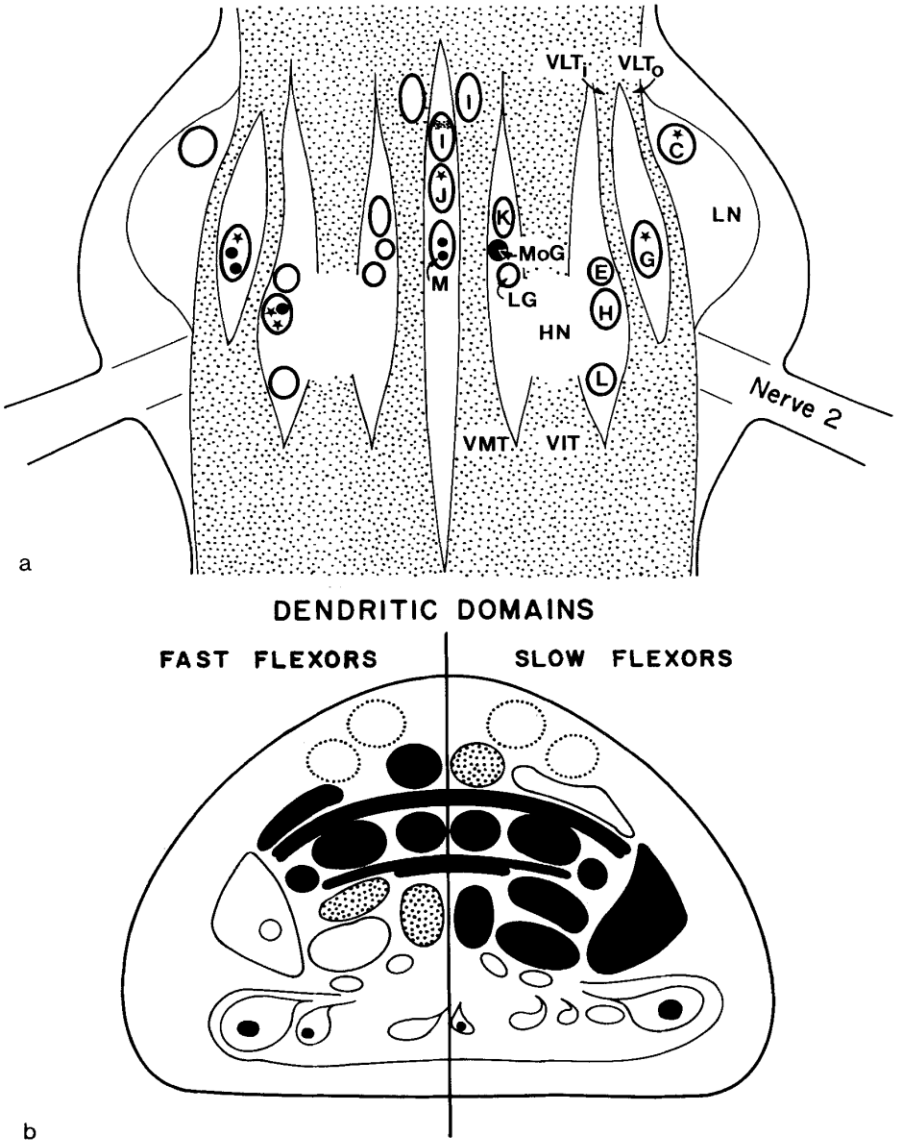


Fig. 25. a. Diagrammatic longitudinal section through the third tract layer. Locations of slow (★)- and fast (●)-flexor linking segments and slow flexor branches (stippling), drawn as though the left N3 were backfilled. Vertical tracts C, E–K, M are labeled according to Kendig ('67). Not all vertical tracts are included. The size of the vertical tracts is exaggerated and axonal positions within a tract are purely illustrative. b. Diagrammatic ganglionic cross section (cf. Fig. 2). Filled structures contain many higher-order dendritic branches. Stippling indicates the presence of only a few branches. The AVC also contains slow-flexor dendrites but is out of the plane of this diagram.

Ganglionic tracts in crayfish and insects

Thoracic ganglia in locusts have large lateral and ventral neuropils whose functions resemble those of the crayfish lateral and horseshoe neuropils. The insect ventral neuropil includes the VAC (ventral association center, Pipa et al., '59; Tyrer and Gregory, '82), the vVAC and aVAC (ventral-most VAC and anterior VAC, Tyrer and Gregory, '82), and the MVAC (medioventral association center, Braunig et al., '81). In insect thoracic ganglia, these neuropils receive input from several types of sensory hairs on the thorax and legs (Anderson, '85b; Johnson and Murphey, '85) and from joint proprioceptors (Braunig et al., '81) and are sites of sensory integration (Siegler and Burrows, '83) that ultimately lead to a variety of motor responses such as grooming (Anderson, '85b), postural changes, or movement (Burrows and Siegler, '84; Burrows and Watkins, '86). The crayfish horseshoe neuropil (HN) is the functional analogue of the insect ventral association centers and also receives afferents from the sensory hairs of the body wall (Leise and Mulloney, '84; unpublished data). These sensory projections will be described in more detail in our next paper (manuscript in preparation). The interneuronal component of this crayfish neuropil is unknown. Interneurons in the nerve cord that can evoke

abdominal flexion or extension have been identified (Larimer and Moore, '84) but their ganglionic neuropilar projections and integrative functions are not fully understood.

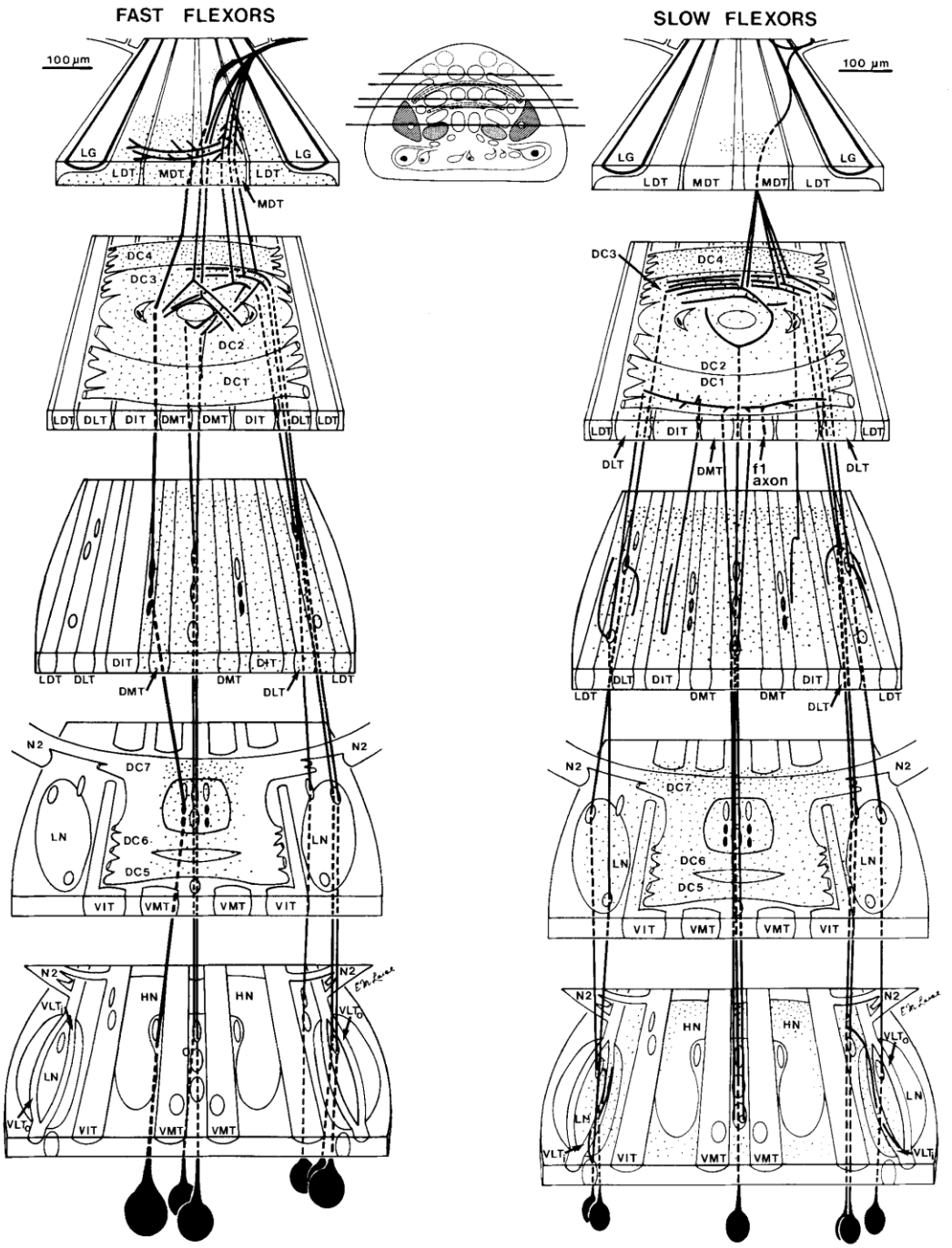


Fig. 26. Main neurites (solid lines), primary branches (solid lines) and some secondary branches of slow- and fast-flexor neurons as they appear in five major levels of an abdominal ganglion. The level of each frontal section is represented by the horizontal lines across the transverse section inserted between the composite drawings. Stippled regions indicate the presence of extensive dendritic branching. For nomenclature of vertical tracts see Figure 25.

The insect lateral neuropils receive proprioceptive input (Braunig et al., '81) and contain branches from local spiking interneurons that integrate afferent sensory information (Burrows and Siegler, '84; Siegler and Burrows, '84; Burrows and Watkins, '86). Many nonspiking local interneurons that control the motor coordination of the legs also branch in these lateral neuropils (Burrows and Siegler, '78; Siegler and Burrows, '79), as do their target motor neurons (Gwilliam and Burrows, '80; Watson et al., '85). In crayfish abdominal ganglia the motor neurons and interneurons of the swimmeret system branch in the lateral neuropils (Leise and Mulloney, '84; Paul and Mulloney, '85). Thus, the lateral neuropils in crayfish and insects are homologous structures concerned with the integration and control of the segmental appendages.

Insect flight neurons also branch in the neuropil in and around the dorsal tracts and extend somewhat into the lateral neuropils (Pearson et al., '85b; Pflüger et al., '86). Both the slow- and the fast-flexor motor neurons in crayfish branch in the neuropil of the dorsal tracts. The flight motor system of insects is believed to be derived from neurons that were once concerned with controlling abdominal musculature. However, until the central projections of motor neurons to the abdominal musculature in insects are described we cannot draw any general conclusions about functional homologies of the dorsal neuropils.

Differences between longitudinal and vertical tracts

We suggest that neurites do not come to lie in particular longitudinal tracts and commissures for the same reasons that they come to lie in particular vertical tracts. Neurons may use vertical tracts because of accidents of position during development, but choose longitudinal tracts to find their appropriate synaptic targets. Both of these factors, soma position and neuronal function, will be important in specifying the shape of a neuron, but we believe that the weight of each factor in a neuron's choice of vertical and horizontal tracts is different.

Crayfish vertical tracts contain mostly main neurites, the linking or integrative segments, that ascend into the ganglionic core (Skinner, '85a). There are vertical pathways in insect ganglia, but the homologues of the crayfish vertical tracts are the radially arranged fiber bundles (Gregory, '74) that carry groups of neurites from adjacent somata into the ganglionic core. Thus, the processes that shape the vertical tracts in crayfish ganglia are probably also the ones that shape the fiber bundles in insects.

If neurons were segregated into tracts by function, one would expect to find slow and fast flexors in different longitudinal and vertical tracts. The linking segments of the fast-flexor motor neurons rise through the ganglia in vertical tracts M, G, and H (Fig. 25), while those of the slow flexors rise in C, G, H, and J. Overlap exists in tracts G and H. Slow and fast flexors also overlap in their use of longitudinal tracts MDT and LDT, through which their axons exit the ganglion. The commissures DC3 and DC2 (Fig. 26) also contain many large branches from both types of flexor neurons. Thus, the segregation by function within the ganglionic tracts is not complete. Factors in addition to function must influence the distribution of neurites within the ganglion.
The position of a neuron's soma when the central nervous system first develops may be one of these factors. During the development of the nervous system, adjacent neurons will send their axons along a pioneered pathway until branch points are reached (Bate and Grunewald, '81; Thomas et al., '84). At the branch points, contact with corner cells or specific subsets of axons within a tract enables differentiating neurons to choose pathways that ultimately lead them to their appropriate synaptic targets (Bate and Grunewald, '81; Taghert et al., '82; Raper et al., '83). Thus, soma position plays a role in dictating which vertical tract a neurite uses to enter a ganglion, but once in the ganglionic core, a neuron's functional connections are more important in specifying the longitudinal tracts, commissures, and neuropils in which it branches.

Another line of evidence supports this view. Insect sensory neurons that are transplanted to a different segment of the body will grow into that segment's ganglion and regenerate their central projections (Murphey et al., '83, '85; Anderson, '85a). These ectopic neurons enter the ganglion through different tracts from those they use in normal development, but they tend to arborize in neuropils homologous to those of their home ganglia (Murphey et al., '83; Anderson, '85a). Often they make functional contacts with appropriate interneurons (Murphey et al., '85). Again, it appears that the route by which a neuron enters a ganglion is of secondary importance, but its branching pattern within the tracts, commissures, and neuropils is critical to its integrative role.

TABLE 1. The Tracts and Commissures That Contain Major Branches of the Fast and Slow-Flexor Motor Neurons

	Fast flexors	Slow flexors
Longitudinal tracts	MDTs, LDTs	MDTs, LDTs, VLTs
Vertical tracts	G, H, M	C, G, H, J
Commissures	DC2, DC3, DC4	DC1, DC2, DC3, DC4

TABLE 2. Dendritic Projections of Fast- and Slow-Flexor Motor Neurons Within the Ganglionic Neuropils[1]

Fast flexors	Slow flexors
—	LNs
—	HN
MDTs, LDTs	MDTs[2]
DMTs, DITs, DLTs	DMTs, DITs, DLTs
VMTs,[2] VITs[2]	VMTs, VITs, VLTs

[1]Dendrites in longitudinal tracts are in TN.
[2]Regions with only a few branches.

Finally, the study of the physiology and morphology of homologous neurons in different ganglia and different species also supports the idea that both positional and functional constraints govern neuronal shape. Pearson et al. ('85a) and Watson et al. ('85) found that the dendritic arborizations varied among segmentally homologous interneurons but that the primary branches were constant. Between species, homologous neurons are usually identified by their physiological properties, common some positions, and branching patterns (Mittenthal and Wine, '78; Paul et al., '85; Sillar and Heitler, '85). Any morphological differences tend to become greater in parallel with physiological changes. For example, the fast-flexor inhibitors in the squat lobster closely resemble those of crayfish in physiology, size, soma position, and general branching pattern (Siliar and Heitler, '85). In contrast, the "MoG" in the squat lobster is unlike its crayfish homologue. It is functionally unspecialized and appears to be a typical fast-flexor motor neuron (Sillar and Heitler, '85). Thus, the putative homology is based only on size and soma position. The "MoG" branches in the ganglionic core, unlike its crayfish counterpart, and lacks the branches and axonal swelling in the connective. From their drawing (Fig. 7, Sillar and Heitler, '85), the "MoG" linking segment appears to rise through the ganglionic core just contralateral to the center, as it does in crayfish (Fig. 26). From these results, we suggest that the dendritic arborizations, longitudinal tracts, and commissural paths used by homologous neurons in different genera are more variable than the vertical tracts through which they enter the ganglion.

Do functionally discrete regions exist in abdominal ganglia?

The dendritic domains of fast- and slow-flexor motor neurons show some spatial overlap but are significantly different (Tables 1, 2). The fast flexors branch mainly in the first and second layers of longitudinal tracts and in both dorsal commissure layers. A few branches reach the third tract layer (Figs. 25, 26). Slow flexors extend into more ganglionic regions: the LNs, the HN, all three commissure layers, and the second and third longitudinal tract layers. A few dendrites reach the first tract layer. The first commissure layer also contains the large primary branches from the slow and fast flexors: slow flexors cross the ganglion in DC1, DC2, DC3, and DC4, fast flexors in DC2 and DC3 (the RT) and possibly in DC4.

If crustacean as well as insect ganglia are organized so that different motor activities are controlled by different areas of neuropil then we would expect to find neurons that are active in only one motor program to have more restricted branching patterns than those involved in several. Altman ('81) proposed this idea for motor neurons with dual functions—those that control the movement of two muscles. If her hypothesis is correct, this prediction should also apply to motor neurons of muscles that are used in more than one behavior. The different arborizations of fast- and slow-flexor motor neurons described here support her idea. The more extensive arborization of the slow-flexor motor neurons within the various ganglionic neuropils thus reflects the more extensive participation of the slow-flexor muscles in the behaviors of these animals.

Site of interactions: Fast flexors

We can predict where information exchange may occur between fast and slow flexors and other components of the abdominal nervous system by examining their branching patterns. Each hemiganglion in Gl-G4 contains two to three fast flexor motor neurons whose axons lie in the next anterior Nerve 3 in *Proc. clarkii* (Mittenthal and Wine, '78). Although we did not fill any of these neurons, we expect their dendritic domains to resemble those of the other fast flexors. In previously published drawings and micrographs, these neurons appear to cross the ganglion in an anterior dorsal commissure (possibly DC1) (Figs. 4 and 5 in Mittenthal and Wine, '78) and inside view do not branch in the ventral two thirds of the ganglion. Selverston and Remler ('72) identified two fast flexors, F8 and F9, in this group, but in their illustrations (Fig. 12 in Selverston and Remler, '72) do not show the axons extending rostrally. The path of their F8 corresponds to that of the anterior neurons described by Mittenthal and Wine ('78), but that of F9 does not. F9 appears to send its integrative segment through a more central pathway in the ganglion. However, drawings of neurons without the surrounding ganglionic structures can be misleading and we make no attempt to alter their descriptions with our information.

The fast-flexor inhibitor receives excitatory input from the muscle receptor organs (Wine, '77b; Edwards and Mulloney unpublished data). Although we did not isolate and fill the fast-flexor inhibitor, data from our fills of stretch receptor axons (Leise and Mulloney, unpublished data) suggest that these synapses occur in the first tract

layer. Similarly, the inhibition of the MoG by command neurons (Wine, '77a) also occurs in the first tract layer. Fast flexors also receive direct excitation from the giant fibers (Wine, '84) and apparent points of contact occur in most fills (Fig. 10). The giant fibers also stimulate a group of interneurons, among which are the segmental giant, SG, and 12 and 13 (Kramer et al., '81; Roberts et al., '82; Kramer and Krasne, '84) that in turn excite fast flexors in bouts of backward swimming, the more flexible "nongiant" tailflips. From the published drawings of these interneurons, we suspect that the SG, 12, 13-fast-flexor synapses occur in both the first tract and commissure layers. The interneurons branch in these same levels and have no other branches that would suggest interactions in other areas.

Sites of interactions: Slow flexors

The slow flexors receive only polysynaptic input from the giant axons (Kuwada and Wine, '79). In none of our preparations did the slow flexors branch near the giant fibers. Swimmerets tend to beat only when the abdomen is extended. The swimmeret neurons branch profusely in the LN (Leise and Mulloney, '84; Paul and Mulloney, '85; Skinner, '85a) and we found that slow flexors also branch here. If there is inhibitory interaction between the swimmeret motor neurons or pattern generators and the slow flexors, it may occur within the LNs.

Slow flexors are also excited by mechanosensory neurons (Kuwada and Wine, '79). Such interactions may occur within the HN. The position of the HN is homologous with that of the insect ventral association center (Gregory, '74) and our axonal fills of sensory neurons in Nerve 2 (Leise and Mulloney, unpublished data) demonstrate that these neurons send many dendrites into the HN. The information exchange occurring within the commissures (DC1-DC7) and the second and third tract layers remain to be determined.

Significance of this organization

Discrete ganglionic neuropils, such as the crayfish LN and HN or the insect VAC, can be thought of as central processing units, analogous to Mountcastle's ('79) cortical modules. This concept of neuropils assumes that the replication of neuropils makes nervous system functions more efficient and that within a neuropil, the close approximation of incoming information and output targets also allows the intervening interneurons to operate efficiently. In vertebrates, these modules are thought to be the basic functional units of the brain. The intrinsic circuitry within each type of neuropil in arthropods might be unique, but any neuropil will receive input, have integrative functions, and send the output to other intraganglionic areas, adjacent ganglia, or the brain. Information transfer between neuropils would occur via interneurons that span large areas of the ganglion (Robertson and Pearson, '83; Paul and Mulloney, '85). In some neuropils all the information received and integrated may be sensory (the HN being one such candidate), but the lack of motor input does not negate the role of the neuropil as a processing unit nor does it negate Altman's hypothesis about ganglionic functions. Finally, we also suggest that not all of the areas of ganglionic neuropil in crayfish should be interpreted as functional units. For example, the wide variety of input to the TN of the dorsal tracts and commissures suggests that these areas may not be organized into distinct units like the LNs or HN.

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